Adhesive and Migratory Behaviors of Nevus Cells Differ from those of Epidermal Melanocytes and are not Linked to the Histological Type of Nevus

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It has been postulated that acquired nevi undergo life span continuous evolution from junctional, presumably in radial expanding phase at the dermal epidermal junction, to compound and then to dermal nested nevi. In an attempt to correlate the morphology of nevi with biological data, we have investigated whether migratory and adhesive phenotypes of nevus cells could account for histological patterns and possible spatiotemporal changes in nevi. Nevus cells were cultured from compound and dermal nevi and compared to normal epidermal cultured melanocytes from children and adults. All nevus cells showed similar *in vitro* adhesive and migratory indexes on laminin-1, laminin-5/nicein, fibronectin, or collagen IV substrates, suggesting that these intrinsic characteristics do not account for the tendency to dermal nesting and/or to radial growth along the dermal-epidermal junction. The cells from epidermal and dermal parts of compound nevi migrated similarly across a reconstituted basement membrane. The results show that intrinsic adhesive and migratory behaviors of nevus cells were not associated with a histological type of nevus. Interestingly, differences in migratory phenotype and intercellular adhesion capacities between nevus cells and normal melanocytes indicated that they could represent different melanocytic cell subpopulations. Finally, melanocytes from adults and children expressed similar levels of the same integrins as all nevus cells but showed differences in function of both α3 and α6 integrin subunits and in migratory/adhesive behaviors, which may suggest different states of melanocyte maturation. *Key words: nevi/melanoma/laminin-5/nicein/integrins.* *J Invest Dermatol 106:1224–1229, 1996*

Whether nevus cells and mature epidermal melanocytes are the same cells is questionable. It is commonly thought, however, that they both originate from the neural crest (Mishima and Schaub, 1966). Nevi are histologically very pleomorphic and have been classified as junctional, compound, or dermal. Because junctional and compound nevi are generally encountered in children and adolescents, while dermal nevi occur more frequently in the elderly, it has been postulated that nevi undergo a continuous evolution from junctional to compound and, finally, to dermal nevi, eventually resulting in their disappearance (Elder and Clark, 1987). In this hypothetical ontogeny of nevi, nevus cells initially proliferate at the dermal-epidermal junction in a lentiginous pattern (lentigo), then tend to form nests and, finally, migrate in the dermis (Elder and Clark, 1987). The so-called "dysplastic nevus," initially defined as a separate nevus (Greene *et al*, 1984), is usually considered as a particular histological pattern of junctional or compound nevi in an active phase of radial growth (Piepkorn, 1990).

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Epidemiological studies have shown extensively that expression of numerous nevi, and particularly the "dysplastic nevi," is a risk factor for melanoma (Greene *et al*, 1984; Grob *et al*, 1990). There is also evidence that acquired nevi can be precursors of melanoma; this event, however, is probably unusual (Kraemer *et al*, 1983; Elder and Clark, 1988). "Dysplastic nevi" could be a step to potential tumor progression from junctional and compound nevi into melanoma (Mishima and Schaub, 1966; Clark, 1991; Shih and Herlyn, 1993). Progression from a purely dermal nevus seems unusual since many melanomas that had been diagnosed as arising in association with dermal remnants of nevus were shown, upon serial step-sectioning, to arise from compound nevi with so-called "dysplastic" features (Friedman *et al*, 1988).

In correlating the morphology of nevi with biological and epidemiological data, it is widely accepted that the junctional lentiginous component represents the "expanding" part of the nevi in radial growth, while dermal nesting may represent nevus cells entering the "quiescent" phase of growth (Clark *et al*, 1984; Piepkorn, 1990). A better knowledge of the biological mechanisms accounting for the different patterns of nevi and/or for their evolution with time is of crucial interest for understanding the development of these benign neoplasms but also, indirectly, for a better assessment of the risk of melanoma. It is tempting to hypothesize that lentiginous expansion, possible migration to dermis and nest formation, may depend on different intrinsic adhesive and migratory properties of nevus cells. In this study we have

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explored *in vitro* the adhesive and migratory behaviors of cultured nevus cells from several types of acquired nevi from different donors as compared to those of normal epidermal cultured melanocytes from adults and children.

MATERIALS AND METHODS

Materials MCDB 153 base powder culture medium and supplements (except epidermal growth factor and antibiotics) as well as collagenase type I were purchased from Sigma (St Louis, MO). Mouse epidermal growth factor was obtained from Collaborative Research (Bedfort, MA). Trypsin, ethylenediamine tetraacetic acid and Dispase grade II were from Boehringer (Mannheim, Germany). Fetal calf serum (FCS) was from Dominique Dutscher (Brumath, France) and bovine pituitaries from Pel-Freez (Rogers, AR). Penicillin/streptomycin solution, pepsinized human placental type IV collagen (cat. 20-70-51-132) and highly purified human fibronectin (cat. 20-70-40-112) were from Jacques Boy (Reims, France). Purified laminin-1 from murine Engelbreth-Holm-Swarm tumor was a kind gift of Dr. Lissitzky (INSERM U387, Marseille, France). Matrigel (growth factors reduced) was purchased from Collaborative Research. Laminin-5/nicein was purified from cultured keratinocyte conditioned medium essentially as already described (Verrando *et al*, 1993). Bovine serum albumin (BSA) and gold chloride ($HAuCl_4$) were from Sigma. Function-blocking monoclonal antibodies to α3 integrin subunit (P1B5) was purchased from Chemicon (Temecula, CA), whereas those to α2 (Gi9), α5 (SAM 1), and α6 (GoH3) were purchased from Immunotech (Marseille, France). The anti-αv integrin subunit was a gift of Dr. Marvaldi (University of Marseilles-Provence, Marseille, France). Control ascite fluid from nonimmune mouse (MOPC 21) was from Sigma. Polyclonal antibody against the S-100 protein, a marker of benign and malignant pigmented tumors, was purchased from Immunotech.

Sample Biopsies Eight compound nevi (nested epidermal and dermal components) and 8 dermal (nested) nevi were obtained from different adult individuals (25–45 years old) at the Dermatology Department, St. Marguerite Hospital, Marseille, France. Informed consent was obtained from each patient after agreement of the committee on ethics. All biopsies were processed for histology. None of these nevi fit the characteristics of the so-called "dysplastic nevi" (Rigel *et al*, 1985). Congenital nevi and "blue nevi" were excluded. The allocation was done by the pathologist, and the portion sent for experimental studies was identified on the pathologic requisition form. Punch biopsies with a diameter identical to those of nevus samples were also taken from each donor from the same geographic area as the nevus biopsy. They were then processed to obtain normal adult epidermal melanocyte cultures. Melanocytes from children were obtained from the foreskins of infants aged from 1 month to 5 years after elective circumcision and placed in culture.

Nevus Cell Cultures After removal of nonlesional subcutaneous tissue and deep dermis, the remaining tissue was cut into 3-mm^2 fragments, washed in calcium-free phosphate-buffered saline (PBS) and incubated for 1 h at 37°C in 0.25% trypsin solution. The epidermis was then removed from the dermis, and the epidermal sheet was digested at 37°C for up to 1 h in a trypsin-ethylenediamine tetraacetic acid solution (0.05–0.02% in PBS), whereas the dermis was digested in PBS containing 1.25 U/ml Dispase grade II plus 0.05% collagenase. Cell suspensions from the epidermis and dermis then were seeded separately in a complete culture medium consisting of MCDB 153 supplemented with 5 μg insulin per ml, 5 ng epidermal growth factor per ml, 16 nM phorbol-12-myristate-13-acetate, 30 μg bovine pituitary extract per ml, prepared according to the procedure of Wilkins *et al* (1985), 2% FCS, and 100 U/50 μg penicillin/streptomycin per ml. Cultures were grown in humidified atmosphere containing 5% CO_2 in air at 37°C. Geneticin (200 μg/ml) was added during the first 2 days of culture to eliminate fibroblast contamination (Halaban and Alfano, 1984). To ascertain the melanocytic phenotype of cultured cells, all of which have dendrites, antibody PEP1 (a kind gift of Dr. V. Hearing, Bethesda, MD) directed against tyrosinase-related-protein-1 and a polyclonal antibody against the S100-protein were used in indirect immunofluorescence technique. All cells expressed both markers. Melanocytic cells in culture originating from the treatment of epidermal portion of skin were considered purely intraepidermal, while those of the dermal portion were considered to originate from the dermal part of the nevi, although there is at present no experimental means of ascertaining this. Contamination of epidermal component cultures from compound nevi by normal epidermal melanocytes from adults was verified by comparing the growth curves of normal epidermal melanocytes and the epidermal component of compound nevi originating from the same skin biopsy surface. From 5-mm-diameter punch biopsies, nevus cells became confluent in 1 wk, whereas melanocytes became confluent in 4 wk after being plated in 2-cm^2 culture dishes. This showed that, during the periods of culture of nevus cells used for our experiments, melanocyte contamination, if any, was negligible.

Normal Melanocyte Culture Cultures of normal melanocytes were settled as already described (Mengeaud and Ortonne, in press).

Flow Cytometry Cells were trypsinized and recovered at 4°C in MCDB 153 medium containing 10% FCS. After centrifugation, the cell pellet was resuspended in MCDB 153 medium containing 1% BSA, and cells were incubated with 5 μg/ml of the respective anti-integrin antibodies (see *Results*) for 2 h at 4°C. Control cells were incubated with the same concentration of immunoglobulins from nonimmune mouse serum (MOPC 21). After washing with PBS, cells were incubated for 45 min at 4°C with fluorescein isothiocyanate- conjugated anti-mouse IgG (diluted 1:500), and were washed twice before fluorescence analysis with an EPICS scan flow cytometer (Coulter, Coultronics France, Margency, France). Five thousand events were acquired, and fluorescence was measured on side and forward scatters in arbitrary units and plotted on a logarithmic scale.

Cell Substrate Adhesion Assay Ninety-six-well plates were coated overnight at 4°C with 50 μl of PBS containing: 1% heat-denatured BSA (control), 30 μg laminin-1 per ml, 3 μg laminin-5/nicein per ml, 20 μg fibronectin per ml, and 20 μg collagen IV per ml. The uncoated sites then were blocked with 1% heat-denatured BSA for 3 h at 4°C. Melanocytic cells were ^{51}Cr-labeled (100 μCi/ml, 3 h), harvested, washed, and resuspended in MCDB 153 plus 1% BSA. One hundred microliters (3,000 cells) were added to the wells, and cells were incubated for 60 min at 37°C in a cell culture incubator. When function-blocking antibodies to integrin subunits were used, they were added during this incubation at final concentrations of 5 μg immunoglobulins anti-α_v per ml, 10 μg P1B5 per ml, 20 μg Gi9 per ml, 20 μg SAM1 per ml, and 20 μg for GoH3 per ml. Negative controls were performed with ascites from a nonimmune mouse (MOPC 21). After three washes with Hanks medium containing 1 mM $CaCl_2$ and $MgCl_2$ to remove the nonadherent cells, the attached cells were lysed in 0.1 M NaOH, and radioactivity was quantitated with a gamma counter. Experiments were performed three times with biopsies from 3 different donors.

Homotypic Intercellular Cell Adhesion Assay Adhesion surfaces were prepared by growing melanocytic cells to confluence in 96-well tissue-culture clusters (Collaborative Research) in nevocyte culture medium. Additional melanocytic cells were ^{51}Cr-labeled (100 μCi/ml, 3 h), detached from culture dishes by incubation with 1.5 mM ethylenediamine tetraacetic acid solution for 15 min at 37°C, and washed in MCDB 153 medium containing 1 mM $CaCl_2$. Cells were then resuspended in the assay medium (MCDB 153 containing 1 mM $CaCl_2$ and 1% BSA). ^{51}Cr-labeled melanocytic cells (3,000) were allowed to adhere to the confluent melanocytic cell layer for 1 h. Nonadherent labeled cells were removed by washing with the MCDB 153-1 mM $CaCl_2$ solution, whereas labeled cells that had bound to the cell layer were dissolved in 0.1 M NaOH and counted in a gamma counter. All experiments were done in triplicate and repeated three times.

Migration Assay A modification of the phagokinetic track assay of Albrecht-Buehler (1977) was used, according to Sarret *et al* (1992). The method was adapted for experiments using 24-well culture plates as described by Verrando *et al* (1994). A gold salt suspension was poured onto glass coverslips at the bottom of the wells. Coverslips were coated overnight at 4°C with 100 μg albumin per ml (control), 3 μg laminin-1 per ml, laminin-5/nicein, 20 μg fibronectin per ml, or 20 μg collagen IV per ml. These concentrations were chosen after preliminary experiments because they were adequately motogenic for easy computer quantification of the tracks. Higher concentrations of laminin-1 (30 μg/ml) did not change the (non-) migratory behavior of the cells.

After removal of the coating medium, 3×10^3 normal melanocytes or nevus cells (primary culture or first subculture) were seeded into their respective wells in a complete nevus culture medium in a cell culture incubator and were allowed to migrate for 4 h. Quantification of the migration was done in triplicate for each experimental condition by computer-assisted image analysis of 3 independent microscopic fields (André *et al*, 1990). Migration was expressed as the percentage of the area of migratory footprints left by the cells on the total surface of the field, and background migration on heat-denatured albumin was subtracted. It was checked over 4 h that no release of ^{51}Cr in the medium occurred from ^{51}Cr-labeled cells, showing that the cells were viable. Using the same culture strain of normal melanocytes at the same passage, we found that the migration index did not vary by more than 20% in different experiments (data not shown).

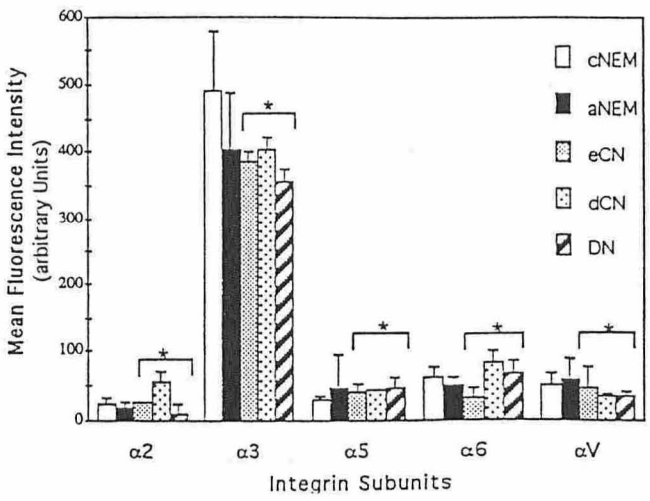

Figure 1. Cell-surface expression of integrin α chains in nevus cells is similar to that of normal melanocytes. Cultured melanocytic cells were trypsinized and reacted with specific integrin α-chain antibodies, then with the corresponding fluoroscein isothiocyanate-conjugated secondary antibody, and fluorescence was determined by flow cytometry. Data are reported as the mean of fluorescence intensity ± SEM (n = 3) (*log scale*, arbitrary units), and experiments were repeated at least twice with three different cultured melanocytic cell strains from three donors. Normal epidermal melanocytes from children (*cNEM*), normal epidermal melanocytes from adults (*aNEM*), epidermal (*eCN*) and dermal (*dCN*) components of compound nevi, and dermal nevi (*DN*). See figure for symbols. *$p > 0.1$ *versus* melanocytes from either adults or children.

Invasion Assay Melanocytic cells were assayed for migration across a filter either coated or not with a matrix of reconstituted basement membrane obtained by deposition of Matrigel. In the former case, both sides of a modified Boyden chamber polycarbonate membrane (Transwell 24, 8-μm pore; Costar, Cambridge, MA) were coated with Matrigel (2 mg/ml) for 2 h at room temperature and washed twice with PBS, and 1×10^5 cells in complete culture medium without FCS were added to each upper chamber unit of the Transwell plate. Lower chambers coated with 1% BSA were filled with complete culture medium without FCS containing 25 μg fibronectin per ml as chemoattractant. The chambers were incubated at 37°C for 48 h, after which the cells were fixed for 10 min in 10% formalin and incubated for 5 min in 0.5% Triton X100. After removal of the melanocytic cells from the upper chamber, nuclei on the bottom were stained with 10 μg propidium iodide per ml. The filters were mounted on glass slides and cells were counted under an epifluorescent microscope. Experiments were done at least three times each on different donors.

Statistical Analysis Statistical significance was measured according to the nonparametric tests of Kruskal-Wallis and Mann-Whitney. Data are expressed as the mean ± SEM.

RESULTS

Nevus Cells from Compound and Dermal Nevi, Like Normal Melanocytes, Express Similar Levels of Integrins, Which Mediate Similar Cell-Substrate Attachment Rates

Cultured nevus cells from both the epidermal and dermal parts of three compound nevi and three dermal nevi were compared to cultured melanocytes from child foreskin and melanocytes from adults.

Figure 1 shows that all melanocytic cells possess integrin subunits α2, α3, α5, α6, and α_v. The α3 integrin was overexpressed in comparison with other subunits, whereas the α2 subunit was underexpressed. No significant differences in expression of these integrin subunits were noticed between epidermal melanocytes from adults or children and nevus cells ($p > 0.1$). In particular, cultured nevus cells from the epidermal part of compound nevi and their dermal counterparts did not differ ($p > 0.1$, not represented on the graph).

Adhesion of melanocytic cells and involvement of the integrin

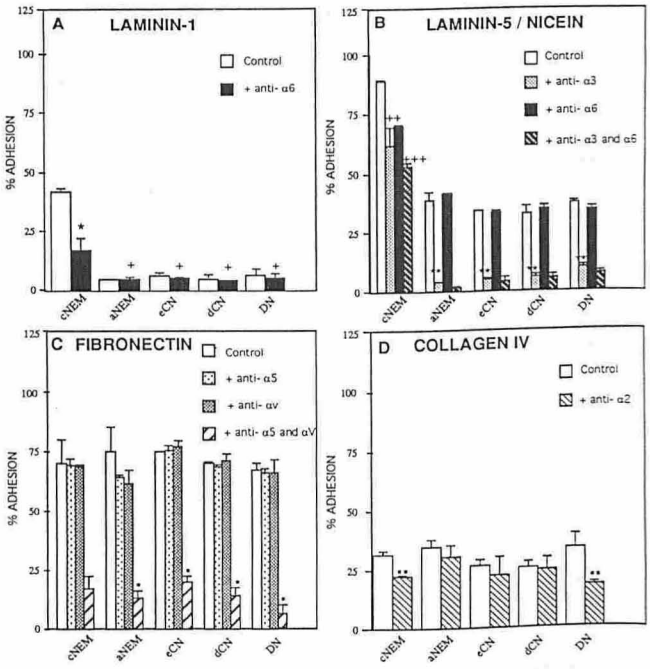

Figure 2. Adhesion of nevus cells to some extracellular matrix components tend to be similar to that of melanocytes from adults. Normal epidermal melanocytes from children (cNEM), normal epidermal melanocytes from adults (*aNEM*), epidermal (*eCN*) and dermal (*dCN*) components of compound nevi, and dermal nevi (*DN*) cultured melanocytic cells were allowed to adhere for 1 h on immobilized extracellular matrix macromolecules in the absence (control) or presence of anti-α6, anti-α3, anti-α5, anti-αv, and anti-α2 (see figure for symbols) integrin function-blocking antibodies. Adhesion is expressed as the percentage of cells from the total initial number of cells in the assay that have adhered. The data are presented as the mean ± SEM (n = 3). These experiments were repeated at least twice with three different melanocytic cultured cell strains from different donors. *$p < 0.01$ *versus* control cNEM; +$p > 0.2$ *versus* respective control nevus cells; **$p < 0.01$ *versus* respective melanocytic cells control from adults; ++$p < 0.04$ *versus* control cNEM; +++$p < 0.01$ *versus* control cNEM; ·$p > 0.2$ *versus* cNEM with function blocking antibodies against α5 and αv; ··$p < 0.05$ *versus* respective control melanocytic cells.

subunits in this process were then studied on different extracellular matrix components in static adhesion assays using function-blocking antibodies to integrin subunits. **Figure 2** shows that similar adhesion rates were observed for all cultured melanocytic cells from adults on a given substrate, i.e., laminin-1, laminin-5/nicein, fibronectin, or collagen IV. Laminin-1, however, did not support high adhesion rates of melanocytic cells from adults, since only 2–8% of nevus cells or melanocytes were able to adhere. Conversely, young melanocytes from child foreskin had an adhesion index 8 times higher on laminin-1 than did melanocytes from adults **(Fig 2*A*)**. In contrast with laminin-1, the other laminin family molecule, laminin-5/nicein, supported the adhesion of all melanocytic cells well **(Fig 2*B*)**. Adhesion of cultured melanocytes from child foreskin was higher (2 times) than that of melanocytic cells from adults.

Adhesion of melanocytic cells to laminin-5/nicein, fibronectin, and collagen IV was mediated by the same ligand-specific integrin subunits, as demonstrated by its inhibition with function-blocking antibodies **(Fig 2)**. In the case of attachment of melanocytes from child foreskin to laminin-1, it could be shown that GoH3 antibody inhibited the adhesion by 60% ($p < 0.01$), indicating that the α6 integrin subunit was the main mediator of this process **(Fig 2*A*)**. No significant inhibition ($p > 0.2$) of the low attachment rate of melanocytes from adults or nevus cells occurred with this antibody, suggesting that α6 was not operating in these cells.

Because P1B5 antibody inhibited 90% of the adhesion ($p < 0.01$)

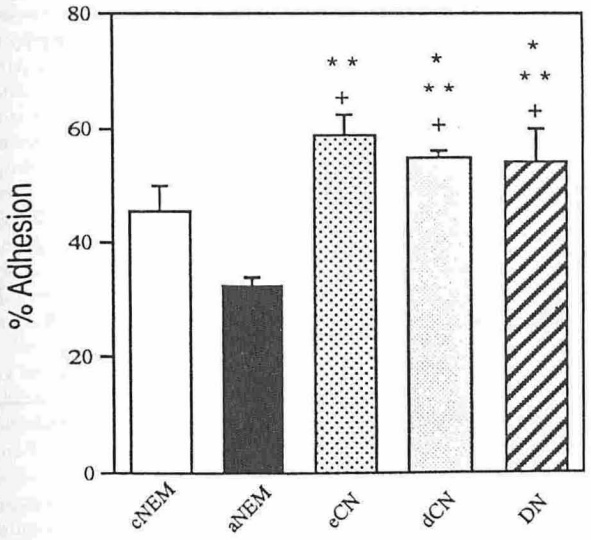

Figure 3. Homotypic intercellular adhesion of nevus cells is higher than that of normal melanocytes. Normal epidermal melanocytes from children (*cNEM*), normal epidermal melanocytes from adults (*aNEM*), epidermal (*eCN*) and dermal (*dCN*) components of compound nevi, and dermal nevi (*DN*) cultured cells were ^{51}Cr-labeled and allowed to adhere for 1 h on confluent layers of respective identical cells. Intercellular adhesion is expressed as the percentage of ^{51}Cr-labeled cells that have adhered on the cell layer from the initial number of ^{51}Cr-labeled cells in the assay. Points are the mean ± SEM of triplicate determinations and have been repeated three times with cells originating from three different donors. *$p > 0.6$ *versus* eCN; **$p < 0.05$ *versus* aNEM; +$p < 0.04$ *versus* cNEM.

of melanocytic cells from adults on laminin-5/nicein, we concluded that the α3 subunit was essentially responsible for cell attachment **(Fig 2*B*)**. Because GoH3 antibody did not block the adhesion of these cells on laminin5/nicein, and combination of P1B5 and GoH3 antibodies did not inhibit it further, we concluded that α6 was not involved in the recognition of laminin-5/nicein in these cells. As melanocytes from child foreskin attached 20% less ($p < 0.04$) in the presence of GoH3 antibody, the α6 subunit was to some extent involved in adhesion on laminin-5/nicein. In this case, synergic inhibition (40%, $p < 0.01$) was obtained when GoH3 and P1B5 were used concomitantly, indicating both α3- and α6-mediated adhesion of melanocytes from children on this substrate.

α5 and αv subunits had to be blocked to prevent melanocytic cell attachment **(Fig 2*C*)**. Both subunits thus equally supported the adhesion to fibronectin. No significant difference ($p > 0.2$) in the participation of these integrins was observed according to the melanocytic cell considered.

Ten to fifty percent of adhesion of melanocytic cells from child foreskin or dermal nevi, respectively, can be inhibited by the antibody Gi9 ($p < 0.05$) on collagen IV **(Fig 2*D*)**. α2 integrin subunits, therefore, only partially mediate the attachment of these cells on collagen IV. In the case of normal melanocytes from adults and cells from compound nevi (epidermal or dermal part), no attachment inhibition was observed in the presence of the Gi9 antibody, indicating that another receptor is involved.

Homotypic Adhesion of Nevus Cells from Compound and Dermal Nevi is Higher than that of Normal Melanocytes We then investigated whether melanocytic cell-cell adhesion differed according to type and localization of nevi and compared to normal epidermal melanocytes. **Figure 3** shows that the ability to form homotypic cell-cell contacts was not significantly different in nevus cells of either compound or dermal nevi ($p > 0.6$). Also, in compound nevi, nevus cells from the epidermal and dermal parts behaved similarly. Interestingly, adhesion of nevus cells was twice ($p < 0.05$) that of normal melanocytes from adults and was even higher (30%, $p < 0.04$) than that of melanocytes from children.

Migration of Cultured Nevus Cells on Extracellular Matrix Components is Similar in Different Histologic types of Nevi but Differs from That of Normal Melanocytes The random migratory behavior of cultured nevus cells from different types of nevi was quantitated on laminin-1, laminin-5/nicein, fibronectin, or collagen IV (haptotactic migration, see *Materials and Methods*). After 4 h, laminin-1 was ineffective in supporting migration of all cultured melanocytic cells tested.

Figure 4 shows the 4-h migration indexes on the other substrates. On laminin-5/nicein, fibronectin, or collagen IV, melanocytes from children always migrated more than those from adults ($p < 0.05$). The lowest migration index was obtained for normal melanocytes from adults. In addition, nevus cells from the dermal part of compound nevi tend to show a higher (2.5 to 3 times) average migration on collagen IV than did their epidermal counterparts ($p = 0.1$). On laminin-5/nicein and fibronectin, migration rates of cells from compound (either epidermal or dermal part) and dermal nevi was not significantly different ($p > 0.7$) but was roughly 2 times higher than that of normal melanocytes from adults ($p < 0.01$ on laminin-5/nicein); this excess migration, however, was not statistically significant on fibronectin ($p = 0.3$).

When the migration rates of cultured nevus cells were compared with cultured melanocytes from epidermis of the same adult donor, identical conclusions could be drawn (data not shown). Differences in the migratory behavior of normal melanocytes *versus* nevus cells could not therefore be attributed to different donors. On the whole, these data indicate that nevus cells had a similar intrinsic migration capacity that was higher than that of normal epidermal melanocytes from adults.

***In Vitro* Invasion Behavior of Cultured Nevus Cells from Compound Nevi Resembles That of Cultured Normal Melanocytes from Adults** To assess the combination of invasion and directed movement of melanocytic cells, the filters of Boyden chambers were coated with Matrigel, which provides basement membrane components, whereas in another set of experiments the filters were not coated to study only directed movement. In the two sets of experiments **(Fig 5)**, melanocytes from children had the highest ability for invasion ($p < 0.004$ for uncoated filters, $p < 0.007$ for coated filters). Normal melanocytes from adults tend to behave like nevus cells ($p = 1$) on uncoated filters (no significant difference occurred between the epidermal and dermal part of compound nevi: $p > 0.1$), but migrated less than nevus cells ($p < 0.007$) on coated filters. No significant difference in migration rates could be detected between the epidermal and dermal part of compound nevi on coated and uncoated filters ($p > 0.1$).

DISCUSSION

The data presented here provide evidence of phenotypic differences between nevus cells and normal melanocytes on the basis of migratory phenotype and intercellular adhesion ability. As compared to normal melanocytes from adults, nevus cells tend to have higher intrinsic migratory and intercellular adhesive capacities. They are also better able to pass through a reconstituted basement membrane made of filters coated with Matrigel; no difference in adhesion to extracellular matrix components was noticed, however, between melanocytes from adults and nevus cells.

Differences in adhesion and migration on extracellular matrix molecules between nevus cells and melanocytes from normal skin were not corroborated by integrin expression, which was found to be similar in all melanocytic cells. They all express α2, the prototype collagen receptor, α3, the prototype laminin-5/nicein receptor, which can also recognize laminin-1, α5, the prototype fibronectin receptor, α6, the prototype laminin-1 receptor, and αv, the prototype vitronectin receptor also binding to fibronectin, von-Willebrand factor, fibrinogen, thrombospondin, and probably laminin-1 (Hynes, 1992), but there is considerable evidence that integrin expression and function are not always correlated. Indeed,

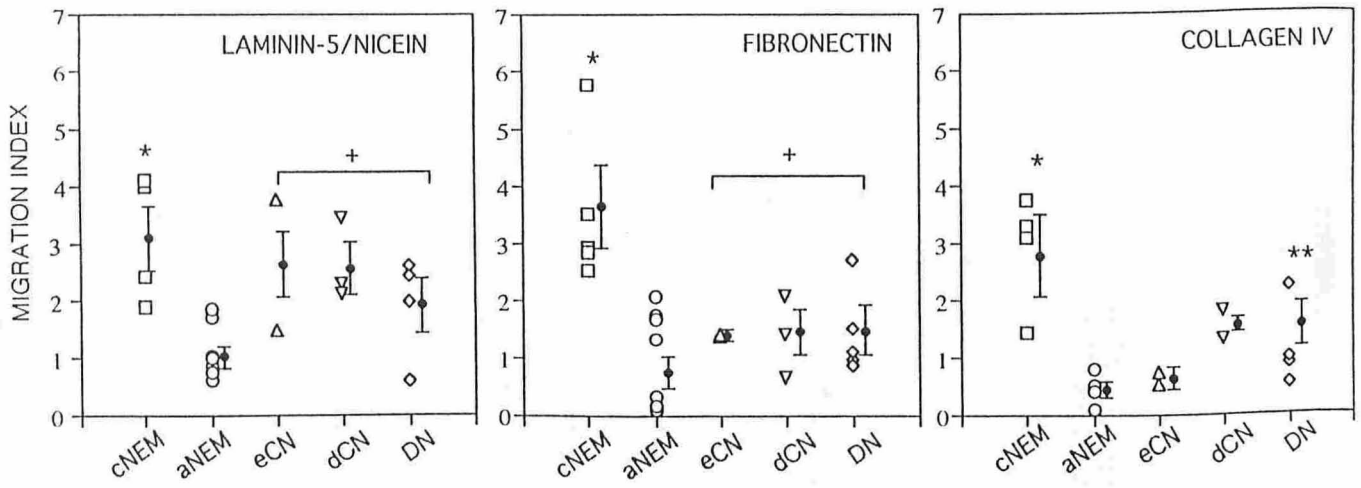

Figure 4. Nevus cells tend to migrate differently to melanocytes on extracellular matrix components. Normal epidermal melanocytes from children (□), normal epidermal melanocytes from adults (○), epidermal (△) and dermal (▽) components of compound nevi, and dermal nevi (◇) cultured cells were assayed for their random haptotactic migration on different immobilized extracellular matrix macromolecules. Points are the mean of three analyzed microscopic fields of cell migrations from different donors in different experiments. For each melanocytic cell type, *black circle points* represent the averaged migration ± SEM from all donors. *$p < 0.05$ *versus* melanocytic cells from adults on laminin-5/nicein; *$p = 0.001$ *versus* melanocytic cells from adults on fibronectin; **$p = 0.1$ *versus* eCN; **$p > 0.35$ *versus* DN; +$p > 0.76$ *versus* all nevus cells, +$p < 0.01$ *versus* aNEM on laminin-5/nicein; +$p = 0.3$ aNEM on fibronectin.

the function is modulated by various mechanisms, including transconformation of the subunits consequent to intracellular signaling involving phosphorylations of their cytoplasmic domains (Kornberg *et al*, 1991) and down regulation (Hotchin and Watt, 1992). Whether the integrin expression *in vitro* reflects the *in vivo* situation is still debated. Albelda *et al* (1990) showed that some differences exist in expression of melanocytic integrins between tissue samples and cultured cells; however, only *in vitro* investigations allow a study of the function of integrins in cell adhesion.

Another result of our investigation is that intercellular adhesion was similar in compound and dermal nevi, but was higher than that in normal melanocytes from adults. This suggests higher expression and/or activation of receptors mediating cell-cell adhesion in nevi. Although we did not examine the nature of such intercellular receptors, a candidate could be the melanoma cell adhesion molecule, Mel-CAM/MUC 18, a 113-kDa glycoprotein of the immunoglobulin superfamily, which is expressed by nevus cells (Nesbit and Herlyn, 1994; Shih *et al*, 1994). Intercellular adhesion molecule 1 is also expressed on benign nevi (Hansen *et al*, 1991; Denton *et al*, 1992) and could be involved in nevus cell-cell adhesion.

The other important result of our biological study is that all nevus cells have similar adhesive and migratory properties *in vitro*. Surprisingly enough, cells from the dermal part of compound nevi migrated more on collagen IV than did their epidermal counterpart. The reason for this remains to be unraveled. In addition, invasion experiments assessing both directed cell movement or combination of cell movement and invasion process also showed that nevus cells from either compartment behaved similarly. Taken together, our findings suggest that these intrinsic characteristics do not account for the tendency to dermal nesting and/or radial growth along the dermal-epidermal junction. Indeed, on the basis of histological and epidemiological data, it was reasonable to hypothesize that nevus cells presenting a lentiginous pattern at the dermal-epidermal junction have a high capacity for migration with weak adhesive properties, conversely to nevus cells located in dermal nests. Nevertheless, other parameters have to be taken into account. First, it could be that lentiginous expansion of compound nevi is the consequence of a high growth capacity, but we did not observe significant variations in growth rates between the different cultured nevus cell strains in culture (data not shown). Second, *in vivo*, nevus cells are submitted to environmental factors such as ultraviolet radiation, keratinocytes, and dermal influences that should play a role in nevus cell behavior.

Finally, our work provides evidence for phenotype difference between melanocytes from children and melanocytes from adults, which may represent a difference in their state of maturation.

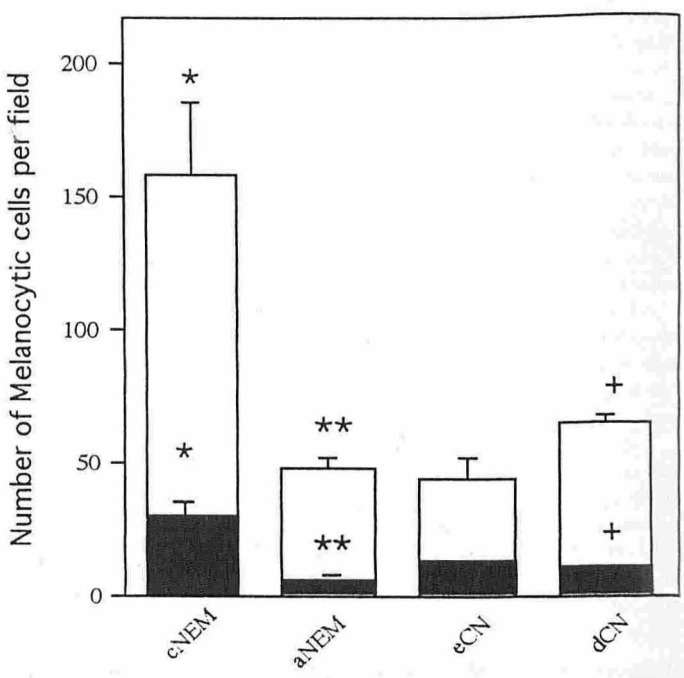

Figure 5. *In vitro* invasion behavior of cultured nevus cells from compound nevi resembles that of normal melanocytes from adults. *White bars*: uncoated Boyden chamber filters. *Black bars*: Boyden chamber filters coated with Matrigel (see *Materials and Methods*). Results are expressed as the mean number of melanocytic cells observed per microscopic field ± SEM *versus* melanocytic cells from adults, *$p < 0.004$ for uncoated filters and *$p < 0.007$ for coated filters. **$p = 1$ *versus* eCN for uncoated filters and **$p < 0.007$ *versus* nevus cells on coated filters. +$p > 0.1$ *versus* eCN.

Melanocytes from adults have a lower adhesion rate on laminins than those from children. They also migrate less on laminin-5/nicein, fibronectin, and collagen IV. Although all melanocytic cells, including nevus cells, express similar levels of integrins, the activation status of α6 and α3 integrins is different in melanocytes from children and adults. On one hand, a partial inactivation of the α6 integrin can be suspected in melanocytes from adults for several reasons. On laminin-1, the lower adhesion rate of melanocytes from adults is not correlated with lesser expression of α6 integrin at the cell surface, and the adhesion can be inhibited by a function-blocking antibody only in melanocytes from children. On laminin-5/nicein, a weak contribution of the α6 integrin subunit to adhesion of melanocytes is observed in children but not in adults. On the other hand, α3 integrin seems to be activated in melanocytes from adults but not from children. This is first supported by the lack of increase in the cell-surface expression of α3 integrin in melanocytes from adults as compared to those from children, and second by the fact that a function-blocking antibody inhibits the adhesion of cells from adults, but not from children, on laminin-5/nicein.

In conclusion, this work provides *in vitro* advances toward an understanding of the physiology of nevus cells in an attempt to correlate histological and epidemiological data.

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